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MICROWAVE STERILIZATION OF TISSUE CULTURE MEDIA

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Keywords: **microwave**-oven, *Populus alba*, *in vitro*, micropropagation

Abstract

Culture media microwaved for 15 min at 390 W or for 5, 10 or 15 min at 520 W gave 96%-100% **sterilization** rates and totally inhibited the growth of *Bacillus subtilis*, *B. megatherium* and *B. coagulans*. Explants of *Populus alba* grown on medium exposed to 260 W **microwave** for 5 min showed the highest frequency of survival and shoot proliferation, while those cultured on medium treated with 520 W for 10 min resulted in the tallest shootlets and greatest number of leaves per shootlet. Although the rooting response and number of roots formed per shoot explant were similar on **microwave**- or autoclave- treated **media**, the longest roots occurred on **media** microwaved for 15 min at 130 W or for 10 or 15 min at 390 W or 520 W.

1. Introduction

It is well known that the major obstacle encountering the use of *in vitro* **culture** techniques is microorganism contamination of **culture media**. Although the **sterilization**

tissue culture media by a **microwave**-oven instead of an autoclave is still limited, the da reviewed by Moore *et al*. (1982) and Wu (1996) affirm that the **microwave** radiation has been found to be more rapid and effective against fungal and bacterial population than steam. Moreover, the microwaved **culture media** can be successfully applied to micropropagation programmes of different species (Tisserat *et al*., 1992). Therefore, in the present paper, attempts were made to determine the influence of various **microwave** powers and times of exposure on **sterilization** of **tissue culture media** and micropropagation behaviour of *Populus alba* explants *in vitro.*

2. Materials and methods

2.1. Plant materials and incubation conditions

The plantlets of *Populus alba* (7-10 cm in height) grown *in vitro* were used as a source of nodal explants. The cultures were incubated in a growth chamber at 24 + 2 under fluorescent light of 3 klux at the level of **culture** jars and at 16-hour photoperiod.

2.2. **Microwave sterilization** of **culture media**

A **microwave**-oven was used for **sterilization** of MS **media** (Murashige and Skc 1962). The **microwave** powers tested were 130, 260, 390 and 520 watts (W). For each power, the three different times of exposure examined were 5, 10 and 15 min. Autoclaving the **media** was applied as control treatment. The microwaved **media** were incubated for three weeks. The bacterial contaminants grown on the microwaved **media** belonged to the genus *Bacillus* according to the morphological tests and the recorded biochemical reactions.

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2.3. Micropropagation trials

At shoot proliferation stage, the nodal explants were vertically cultured on the fu strength MS medium and incubated for six weeks. Then, the survival rate, number of shootlets proliferated per explant, length of the shootlets and number of leaves formed pe shootlet were recorded.

At the rhizogenesis stage, the microshoots resulting from a shoot proliferation stage were aseptically excised and vertically cultured for six weeks on the half strength MS-medium enriched with 5 mg/l IBA. The rooting rate, number of roots formed per

MS medium enriched with 3 mg/l IBA. The rooting rate, number of roots formed per shoot explant and length of roots (cm) were measured.

2.4. Statistical analysis

The statistical lay-out of the experiments was completely randomized design. Th percentages were transformed into angular values (arcsin ϖ%) and tested by analysis of variance. LSD test was used for comparison of means.

3. Results and discussion

3.1. **Sterilization** of **culture media**

As shown in Table 1, the **culture media** microwaved for 15 min at 390 W or for 10 or 15 min at 520 W exhibited the highest **sterilization** efficiency (96%-100%). However, **culture media** microwaved for 5 min at 130 W resulted in the lowest value (36% of **sterilization**). These data prove that a micowave-oven can be used alternatively to an autoclave for **culture media sterilization**. These findings coincide with those detected by Moore *et al*. (1982).

Data in Table 1 reveal that increasing the time of exposure for all **microwave** powers reduced significantly the growth of all bacteria isolated. A complete growth inhibition of *B. subtilis* and *B. coagulaus* was found when **media** were exposed to 390 W for 5 min and 520 W for 10 or 15 min. *B. megatherium* was the most sensitive strain to **microwave** treatments. The lethal effect of **microwave** radiation against several bacterial strains has been affirmed by Rosaspina *et al*. (1994) and Wu (1996).

3.2. Micropropagation behaviour of *Populus alba* explants *in vitro*

The explants grown on **media** exposed to 260 W **microwave** for 5 min showed t highest frequency of survival and shootlets proliferation, whereas those cultured on **medi** treated with 520 W for 10 min gave the tallest shootlets and greatest number of leaves pe shootlet (Table 2). This indicates that the physiological activities accompanying shoot proliferation stage of micropropagation might be favoured in case of using microwaved **media** as compared to autoclaved ones. Contrarily, Tisserat *et al*. (1992) observed that th growth rate of *Fragaria vesca* shootlets cultured on either microwaved or autoclaved **media** was similar.

Both the **microwave**- or autoclave- treated **media** had an equal effect on the rooting rate of shootlet explants and number of roots formed per shootlet. **Media** microwaved for 15 min at 130 W or for 10 or 15 min at 390 W or 520 W produced the longest roots (Table 2).

References

Moore, G.M., Hall, R.G. and James, E.A. 1982. Pasteurization of growing **media** by **microwave** radiation. Proc. Int. Plant Propagators Soc. 32:45-55.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco **tissue culture**. Physiol. Plant. 15:473-497.

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Rosaspina, S.; Liguori, G., Anzanel, D., Fenzi, G. and Salvatorelli, G. 1994. Experimenta tests of a **microwave sterilization** system. Minerva Stomatal 43:17-21

Tisserat, B., Jones, D. and Gallette, P.D. 1992. **Microwave sterilization** of plant **tissue culture**. Hortiscience 27:358-361

Wu, Q. 1996. Effect of high-power **microwave** on indicator bacteria for **sterilization**. Trans Biomed Eng. 43:752-754.

Tables

Table 1. Effect of **microwave** treatments on tisssue **culture media sterilization**.

	Sterilization effeciency		
Treatments	%	Arcsin %	Survival rate of bacteria
Autoclave	100	90.00	Non
Microwave			
Power (watts)	Time (min)		
130	5	36	36.18	*Bacillus subtilis* (10
130	10	48	43.79	*B. subtilis* (10
130	15	40	38.91	*B. subtilis* (80%) and *B. megatherium* (
260	5	66	54.51	*B. subtilis* (10
260	10	90	75.69	*B. subtilis* (10
260	15	88	72.00	*B. subtilis* (33%) and *B. coagulans* (67
390	5	88	74.36	*B. subtilis* (10
390	10	90	75.69	*B. subtilis* (33%) and *B. coagulans* (67
390	15	100	90.00	Non
520	5	96	82.62	*B. subtilis* (10
520	10	100	90.00	Non
520	15	100	90.00	Non
L.S.D. 5%		12.21		
L.S.D. 1%		17.48		

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Table 2. Effect of **microwave** treatments on micropropagation of *Populus alba*.

Treatments	Survival rate		Shoot formation		
	%	Arcsin %	Number of shootlets per explant	Length of shootlets (cm)	Number of leaves per shootlet
Autoclave	85	66.42	2.19	7.60	7.73
Microwave Power (watts)	Time (min)				
130	5	62	51.94	2.62	9.39	8.38
	10	72	58.05	2.13	5.99	6.15
	15	84	64.20	2.24	7.93	7.34
260	5	96	78.46	2.75	8.94	6.95
	10	76	60.57	1.56	8.51	7.37
	15	66	54.33	1.38	7.40	7.16
390	5	92	73.57	1.98	6.27	6.52

10	74	59.34	2.05	7.69	6.34
15	72	58.05	1.91	9.22	6.74
520 5	94	75.82	1.37	10.67	6.77
10	80	63.44	1.53	12.81	8.67
15	74	59.34	1.67	8.96	7.70
L.S.D. 5%		13.90	0.71	2.66	1.40
L.S.D. 10%		19.93	0.95	3.55	1.86

NS Means are not significantly different.

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